REMARKS

Favorable consideration and allowance are respectfully requested for claims 1-16 in view of the foregoing amendments and the following remarks.

Responsive to the restriction requirement seeking restriction between the sequences listed in the claims, reconsideration and withdrawal of the requirement are respectfully requested.

This requirement is traversed because polypeptide or protein sequences meet the requirements set forth in *In re Harnish* for a proper Markush group in that they (1) share a common utility and (2) share a substantial structural feature essential to that utility. With regard to the first factor of common utility, all of the protein sequences are described as sodium-dependent phosphate co-transporters. With regard to the second factor, all of the protein sequences exhibit an extremely high degree of sequence identity. SEQ ID NO:2 differs from SEQ ID NO:4 by only one single amino acid out of 560 (Ala at position 327 instead of Asp). Similarly SEQ ID NO: 6 differs from SEQ ID NO:8 by only a single amino acid out of 560 (Val at position 528 instead of Ala). SEQ ID NO:6 differs from SEQ ID NO:2 by only 9 amino acids of 560, and SEQ ID NO:8 differs from SEQ ID NO:2 by only eight amino acids out of 560. Similarly SEQ ID NO: 12 differs from SEQ ID NO:14 by only two amino acids out of 582 (Ser at position 568 instead of Gly, and Ser at position 574 instead of Thr) and from SEQ ID NO:10 by only 13 amino acids out of 582. SEQ ID NO:10 is 100% identical to SEQ ID NO:2 over the 60 amino acid stretch from Gly410 to Lys469 (amino acids 402 to 461 of SEQ ID NO:2), 100% identical to SEQ ID NO:2 over the 44 amino acid stretch from Phe306 to Gly349 (amino acids 298 to 341 of SEQ ID NO:2, and 100% identical to SEQ ID NO:2 over the 56 amino acid stretch from Thr162 to Thr217 (amino acids 154 to 209 of SEQ ID NO:2), as well as being approximately 88% identical over the 458 amino acid stretch from Asp61 to Phe518 (amino acids 53 to 510 of SEQ ID NO:2). The claimed proteins encoded by the polynucleotides of SEQ ID NOs: 1, 3, 5, 7, 9, 11 and 13 are the respective

polypeptides of SEQ ID NOs: 2, 4, 6, 8, 10, 12 and 14. In view of this common utility and common structure, a Markush grouping of the respective protein sequences is believed proper, and the provisions of M.P.E.P. §803.02 are applicable. Therefore, the Examiner is respectfully requested to withdraw this restriction requirement and treat the foregoing provisional election of SEQ ID NO:4 as an election of species in accordance with the provisions of M.P.E.P. §803.02 which govern the examination of Markush claims containing patentably distinct species.

The rejection of claims 1-4, 7-12 and 14-15 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement for a method for detecting a pain-regulating substance is respectfully traversed.

The standard for adequate enablement is whether the specification describes the claimed subject matter in such a way as to enable a person skilled in the art to which it pertains to use the invention. Thus, enablement is judged in view of the combined teachings of the specification and the knowledge of one skilled in the art.

The specification describes that the inventors undertook to identify pain-regulated genes which are modified in their expression under pain conditions and that these genes are thought to be involved in the development and processing of chronic pain, see paragraph [0007] of the specification. Upon identification of these genes, methods of detecting pain-regulating substances may be performed by testing potential pain-regulating substances for its capacity to bind the protein produced by these genes or by measuring functional parameters modified by the binding of the test substance.

The specification describes numerous tests which were performed in order to identify certain proteins as relevant to pain. These include tests related to CFA-inducted polyarthritis (beginning at paragraph [0086]); CIA-induced arthritis (beginning at paragraph [0099]); as well as others identified in the Examples provided in the specification. The abstracts of certain available literature are provided as Appendix A in further support of Applicant's position that the claims are properly enabled.

The U.S. Court of Customs and Patent Appeals has stated that "The first paragraph of § 112 requires nothing more than objective enablement. How such a teaching is set forth, either by the use of illustrative examples or by broad terminology, is of no importance." *In re Marzocchi*, 169 USPQ 367 , 369 (CCPA 1971). The court also added that "it is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure." *In re Marzocchi*, 169 USPQ 367 , 370 (CCPA 1971). The present record includes no such statement or other explanation as to why the truth of the accuracy of disclosure statements should be doubted.

There is nothing in the record to suggest any reason why the method would not work as claimed. Indeed, based on the knowledge of a person of skill

in the art and the evidence provided in the Examples provided in the present specification, a person of ordinary skill in the art would conclude that all of the claimed method would be operable.

As indicated above, the burden is on the Patent Office to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. On the present record there is no such explanation, and no apparent reason is offered to support the notion that the statements in the specification are not true or accurate. Moreover, the present record provides ample evidence that the claimed compounds would be active for the claimed methods.

The Office Action states that "the method is directed to 'A method for detecting a pain-regulating substance' *which cannot be measured* by the instant method. In this respect, it is important to note that the method is directed to detecting a pain-regulating substance, not measuring the degree to which a substance may regulate pain. Accordingly, there is no requirement that the claim actually provide steps that allow for the measurement of pain. The relevance of the substance to pain is provided through the substance's capacity to bind proteins which are modified in their expression under pain conditions, or modify other functional parameters by their binding. No further experimentation is required to measure the pain

For the foregoing reasons, a person of skill in the art would be able to practice the claimed invention without further undue experimentation. Accordingly, reconsideration and withdrawal of the rejection of claims under 35 U.S.C. § 112, first paragraph, are respectfully requested.

The rejection of claims 1-4, 7-12 and 14-15 under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement is respectfully traversed.

As amended, claim 1 further clarifies that the functional parameter modified by binding is determined "via measurement of the regulation, inhibition or activation of receptors, ion channels or enzymes or via measurement of a modification in gene expression, ionic medium, pH or membrane potential, or via a modification in enzyme activity or concentration of a second messenger." Support for this amendment is provided in the specification, at least at page 23, second paragraph.

Accordingly, the claim is believed to be definite, as the particular functional parameters are laid out in the claim.

The Office Action notes that claims 11 and 12 recite certain measurements, however the Office Action indicates that the specification fails to disclose sufficient measurements for any protein. To satisfy the written description requirement, a patent specification must describe the claimed

invention in sufficient detail so that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention. See, e.g., *Moba, B.V. v. Diamond Automation, Inc.*, 325 F.3d 1306, 1319, 66 USPQ2d 1429, 1438 (Fed. Cir. 2003). An applicant shows possession of the claimed invention by describing the claimed invention with all of its limitations using such descriptive means as words, structures, figures, diagrams, and formulas that fully set forth the claimed invention. *Lockwood v. American Airlines, Inc.*, 107 F.3d 1565, 1572, 41 USPQ2d 1961, 1966 (Fed. Cir. 1997).

Methods to provide the recited measurements are all widely known to persons of skill in the art. Specific examples for proposed procedures are provided in the specification at least in Examples 5 and 6. This disclosure adequately shows possession of the claimed invention. Accordingly, there is no need to provide specific measurements. The lack of such measurements does not show, or even suggest that the present Applicants were not in possession of the claimed invention.

The remaining claims are all dependent from claim 1 include the limitations introduced by this amendment.

The Office Action asserts that "only measurement of the function of the isolated polypeptide of SEQ ID NO: 4 meets the written description requirement." Applicants wish to point out that the claim involves simply incubating a test substance with a biomolecle and then measuring binding of the test substance to that biomolecule or measuring a functional parameter modified by this binding. As amended, claim 1 defines the particular functional parameters that are measured. Further, the invention is described in detail in the specification. Given the similarities of the disclosure for the various proteins and as well as the claim language directed to the homologous proteins embraced by the claim, the indication that the written description requirement is met for

the protein of SEQ ID NO: 4 but not the other proteins is an improper application of the law. As stated before, there is no requirement in the patent laws that examples be provided. A person of skill in the art would clearly understand that the applicants were in possession of the invention as defined by the claims. Therefore, reconsideration and withdrawal of this rejection are therefore respectfully requested.

The rejection of claims 1-4, 7-12 and 14-15 under 35 U.S.C. § 112, second paragraph, as indefinite, is respectfully traversed.

The Office Action asserts that the phrase "a method of detecting a pain-regulating substance" means detecting a potential regulating influence. However, because of the relationship between binding of the test substance and pain regulation, the method is actually directed to methods of detecting pain-regulating substances. On page 9 the specification indicates that pain regulating means directly or indirectly influencing the perception of pain. This characteristic is suitably determined by the binding or functional properties measured by the claimed method. A person of skill in the art would have no trouble in determining the scope of the claim. Accordingly, the claim language accurately and definitely describes the claimed invention.

The Office Action asserts that the phrase "stringent conditions" is not defined in the specification. Applicants note, however that the phrase is particularly defined on page 16 of the specification. In particular, the specification indicates that stringent conditions are those conditions under which

only perfectly base-paired nucleic acid strands are formed and remain stable. A person of skill in the art would understand the reference to "binds under stringent conditions" to refer to those nucleic acid strands that are perfectly base paired. One of skill in the art could readily determine whether nucleic acid strands are perfectly base paired and the scope of the claim is therefore definite.

The Office Action also asserts that claim 1 is indefinite because it is unclear whether step (b) is practiced with the biomolecule of step (a) or only with a cell synthesizing the biomolecule. Step (b) recites an "or" between these alternative for measuring and accordingly, a person of skill in the art would understand that the claim contemplates methods including either of these alternatives.

The Office Action also asserts that claim 1 is indefinite because it omits a step of "determining if the compound has detected a pain regulating substance." Based on the relationship between the proteins recited as falling within Group I, and the knowledge that these proteins are pain-relevant, a person of skill in the art would expect that the compounds identified to bind the proteins or to modify one of the measured functional parameters will be pain regulators. Accordingly, this allegedly missing step is provided in the method.

The Office Action cites to § 2172.01 of the MPEP, however this section appears irrelevant. The portion of § 2172.01 relevant to indefiniteness is as follows:

> In addition, a claim which fails to interrelate essential elements of the invention as defined by applicant(s) in the specification may be rejected under 35 U.S.C. 112, second paragraph, for failure to point out and distinctly claim the invention. See *In re Venezia*, 530 F.2d 956, 189 USPQ 149 (CCPA 1976); *In re Collier*, 397 F.2d 1003, 158 USPQ 266 (CCPA 1968). >But see *Ex parte Nolden*, 149 USPQ 378, 380 (Bd. Pat. App. 1965) ("[I]t is not essential to a patentable combination that there be interdependency between the elements of the claimed device or that all the elements operate concurrently toward the desired result"); *Ex parte Huber*, 148 USPQ 447, 448-49 (Bd. Pat. App. 1965) (A claim does not necessarily fail to comply with 35 U.S.C. 112, second paragraph where the various elements do not function simultaneously, are not directly functionally related, do not directly intercooperate, and/or serve independent purposes.)

This portion of the MPEP relates to interrelating essential elements and says nothing about a requirement that method claims repeat the language of the preamble. The remainder of this section of the MPEP relates to enablement rejections. Accordingly, this section of the MPEP is irrelevant and reconsideration and withdrawal of this rejection are respectfully requested.

The Office Action also asserts that claim 1 is indefinite for using the term "functional parameter". As amended the functional parameter is specifically defined in claim 1. In view of this amendment, reconsideration and withdrawal of this rejection are respectfully requested.

Claims 2-4 and 7 are alleged to be indefinite for reciting "genetic engineering." This phrase is commonly known and widely used by persons of skill in the art. A search of the electronic records available on the U.S. Patent and Trademark Office website indicates that since 1976, the phrase "genetic engineering" appears in the claims of seventy issued patents. Further, the phrase is defined on page 21, near the beginning of paragraph [0019]. Accordingly, a person of skill in the readily would readily understand the

meaning of this claim term and reconsideration and withdrawal of the rejection are respectfully requested.

Claim 4 is allegedly indefinite for reciting expression of a G protein. The meaning of the term G protein is provided in the specification on page 21 which indicates that it is a GTP-binding protein. The Office Action asserts that it is not evident how this limitation of claim 4 relates to the claimed method. Reviewing the claims, they clearly show how the limitation relates to the claimed method. In particular, the claims states that the manipulation by genetic engineering of claim 3 causes expression of a form of a G protein. Claim 4 depends from claims 1-3, which recite that the cell is manipulated by genetic engineering and that this allows for the measurement of at least one functional parameter modified by the binding of the test substance.

A person of skill in the art could readily determine the scope of claim 4, and accordingly it meets the requirements for definiteness. Reconsideration and withdrawal of the rejection are respectfully requested.

Claim 9 is allegedly indefinite for reciting a "native" mammalian cell. As amended, the word "native" is deleted. Thus, the objected-to phrase does not appear in the claim and reconsideration and withdrawal of this rejection are respectfully requested.

The rejection of claims 1-3, 7-12 and 14 under 35 U.S.C. § 102(e) as anticipated by Edwards et al. (US 2002/0098473) is respectfully traversed.

The provisional application from which the cited reference claims priority, does not appear to contain the portions of the reference disclosure relied on in the Office Action. The provisional application does not mention pain or management of pain in any way. The provisional application also does not disclose the so-called VGLUT enzyme. Accordingly, for purposes of information relied on in the rejection, the reference would not be entitled to an effective date earlier that its July 24, 2001 actual U.S. filing date.

The present application claims priority to German Application No. 101 28 541.8 which was originally filed June 13, 2001. Accordingly, 102(e) date of the reference postdates the invention date of the present application and the anticipation rejection cannot, therefore, be properly maintained. A certified translation of the priority document is submitted herewith. Reconsideration and withdrawal of this rejection are respectfully requested.

CONCLUSION

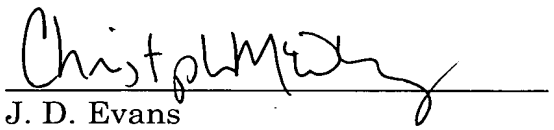
In view of the foregoing, the application is respectfully submitted to be in condition for allowance, and prompt, favorable action thereon is earnestly solicited.

If there are any questions regarding this amendment or the application in general, a telephone call to the undersigned would be appreciated since this should expedite the prosecution of the application for all concerned.

Although an Extension of Time is submitted herewith, if necessary to effect a timely response, this paper should be considered as a petition for an Extension of Time sufficient to effect a timely response, and please charge any deficiency in fees or credit any overpayments to Deposit Account No. 05-1323 (Docket #029310.52995US).

Respectfully submitted,

December 14, 2005



J. D. Evans
Registration No. 26,269

Christopher T. McWhinney
Registration No. 42,875

CROWELL & MORING LLP
Intellectual Property Group
P.O. Box 14300
Washington, DC 20044-4300
Telephone No.: (202) 624-2500
Facsimile No.: (202) 628-8844
JDE:CTM:mdm
2653848

Appendix A

Literature Relevant to Enablement

Hwang SJ, Burette A, Rustioni A, Valtschanoff JG.
Vanilloid receptor VR1-positive primary afferents are glutamatergic and contact spinal neurons that co-express neurokinin receptor NK1 and glutamate receptors. J Neurocytol. 2004 May;33(3):321-9.

Li JL, Fujiyama F, Kaneko T, Mizuno N.
Expression of vesicular glutamate transporters, VGluT1 and VGluT2, in axon terminals of nociceptive primary afferent fibers in the superficial layers of the medullary and spinal dorsal horns of the rat. J Comp Neurol. 2003 Mar 10;457(3):236-49.

1: Neuropharmacology. 2005 Aug 16; [Epub ahead of print] Related Articles, Links

Selective cortical VGLUT1 increase as a marker for antidepressant activity.

Moutsimilli L, Farley S, Dumas S, El Mestikawy S, Giros B, Tzavara ET.

INSERM U513, Neurobiologie et Psychiatrie, 8, rue du General Sarrail, Creteil 94010 cedex, France.

The two recently characterized vesicular glutamate transporters (VGLUT) presynaptically mark and differentiate two distinct excitatory neuronal populations and thus define a cortical and a subcortical glutamatergic system (VGLUT1 and VGLUT2 positive, respectively). These two systems might be differentially implicated in brain neuropathology. Still, little is known on the modalities of VGLUT1 and VGLUT2 regulations in response to pharmacological or physiological stimuli. Given the importance of cortical neuronal activity in psychosis we investigated VGLUT1 mRNA and protein expression in response to chronic treatment with commonly prescribed psychotropic medications. We show that agents with antidepressant activity, namely the antidepressants fluoxetine and desipramine, the atypical antipsychotic clozapine, and the mood stabilizer lithium increased VGLUT1 mRNA expression in neurons of the cerebral cortex and the hippocampus and in concert enhanced VGLUT1 protein expression in their projection fields. In contrast the typical antipsychotic haloperidol, the cognitive enhancers memantine and tacrine, and the anxiolytic diazepam were without effect. We suggest that VGLUT1 could be a useful marker for antidepressant activity. Furthermore, adaptive changes in VGLUT1 positive neurons could constitute a common functional endpoint for structurally unrelated antidepressants, representing promising antidepressant targets in tracking specificity, mechanism, and onset at action.

PMID: 16111724 [PubMed - as supplied by publisher]

2: Arch Ital Biol. 2005 May;143(2):127-32. Related Articles, Links

Heterogeneity of axon terminals expressing VGLUT1 in the cerebral neocortex.

Conti F, Candiracci C, Fattorini G.

Department of Neuroscience, Section of Physiology, Universita Politecnica delle Marche, Via Tronto 10/A, Torrette di Ancona, 1-60020 Ancona, Italy. f.conti@univpm.it

Using immunocytochemical techniques and confocal microscopy we have studied the localization of the vesicular glutamate transporters (VGLUTs) 1 and 2 in the mammalian cerebral cortex. The cardinal observations gathered to date can be summarized as follows: 1) Many VGLUT1-positive puncta coexpressing synaptophysin-1 outline pyramidal cell somata and proximal dendrites; of these, a sizeable fraction coexpress VGAT, the vesicular transporter for GABA; 2) VGLUT2-positive puncta are also present in layers II-III and some of them coexpress VGLUTI. These findings suggest that in the cerebral cortex of adult rats axon terminals expressing VGLUT1 are heterogeneous.

PMID: 16106993 [PubMed - indexed for MEDLINE]

3: Brain Res. 2005 Sep 7;1055(1-2):122-30. Related Articles, Links

Bilateral enhancement of excitation via up-regulation of vesicular glutamate transporter subtype 1, not subtype 2, immunoreactivity in the unilateral hypoxic epilepsy model.

Kim DS, Kwak SE, Kim JE, Won MH, Choi HC, Song HK, Kwon OS, Kim YI, Choi SY, Kang TC.

Department of Anatomy, College of Medicine, Hallym University, Chunchon, Kangwon-Do 200-702, South Korea.

In the present study, the change of vesicular glutamate transporter (VGLUT) immunoreactivity on long-term impaired excitability in the hippocampus after recovery from unilateral hypoxic-ischemic insult was investigated in order to extend our understanding of the mechanism of epileptogenesis using unilateral hypoxic epilepsy models. Both the lesioned (submitted to ischemia) and the unlesioned hippocampi exhibited the frequent occurrence of interictal spikes and occasionally the sustained ictal discharges. However, paired-pulse inhibition was significantly reduced in the unlesioned dentate gyrus, not in the lesioned dentate gyrus. VGLUT1 immunoreactivity was significantly elevated in both hippocampi following hypoxic ischemia, although VGLUT2 immunodensity was unaltered. These findings suggest that the enhancement of VGLUT1 immunoreactivity in both hippocampi after unilateral hypoxic ischemia may contribute to the hyperexcitability, which may play an important role in the epileptogenesis (presumably accompanied by altered inhibitory transmission) after neurodegeneration.

PMID: 16083865 [PubMed - in process]

4: J Neurosci. 2005 Aug 3;25(31):7121-33. Related Articles, Links

Homeostatic scaling of vesicular glutamate and GABA transporter expression in rat neocortical circuits.

De Gois S, Schafer MK, Defamie N, Chen C, Ricci A, Weihe E, Varoqui H, Erickson JD.

Neuroscience Center of Excellence, Louisiana State University Health Sciences Center, New Orleans, Louisiana 70112, USA.

Homeostatic control of pyramidal neuron firing rate involves a functional balance of feedforward excitation and feedback inhibition in neocortical circuits. Here, we reveal a dynamic scaling in vesicular excitatory (vesicular glutamate transporters VGLUT1 and VGLUT2) and inhibitory (vesicular inhibitory amino acid transporter VIAAT) transporter mRNA and synaptic protein expression in rat neocortical neuronal cultures, using a well established in vitro protocol to induce homeostatic plasticity. During the second and third week of synaptic differentiation, the predominant vesicular transporters expressed in neocortical neurons, VGLUT1 and VIAAT, are both dramatically upregulated. In mature cultures, VGLUT1 and VIAAT exhibit bidirectional and opposite regulation by prolonged activity changes. Endogenous coregulation during development and homeostatic scaling of the expression of the transporters in functionally differentiated cultures may serve to control vesicular glutamate and GABA filling and adjust functional presynaptic excitatory/inhibitory balance. Unexpectedly, hyperexcitation in differentiated cultures triggers a striking increase in VGLUT2 mRNA and synaptic protein, whereas decreased excitation reduces levels. VGLUT2 mRNA and protein are expressed in subsets of VGLUT1-encoded neocortical neurons that we identify in primary cultures and in neocortex in situ and in vivo. After prolonged hyperexcitation, downregulation of VGLUT1/synaptophysin intensity ratios at most synapses is observed, whereas a subset of VGLUT1-containing boutons selectively increase the expression of VGLUT2. Bidirectional and opposite regulation of VGLUT1 and VGLUT2 by activity may serve as positive or negative feedback regulators for cortical synaptic transmission. Intracortical VGLUT1/VGLUT2 coexpressing neurons have the capacity to independently modulate the level of expression of either transporter at discrete synapses and therefore may serve as a plastic interface between subcortical thalamic input (VGLUT2) and cortical output (VGLUT1) neurons.

PMID: 16079394 [PubMed - in process]

5: J Neurochem. 2005 Aug;94(4):875-83. Epub 2005 Jun 30. Related Articles, Links

Evidence for increased expression of the vesicular glutamate transporter, VGLUT1, by a course of antidepressant treatment.

Tordera RM, Pei Q, Sharp T.

University Department of Pharmacology, Oxford, UK.

The therapeutic effect of a course of antidepressant treatment is believed to involve a cascade of neuroadaptive changes in gene expression leading to increased neural plasticity. Because glutamate is linked to mechanisms of neural plasticity, this transmitter may play a role in these changes. This study investigated the effect of antidepressant treatment on expression of the vesicular glutamate transporters, VGLUT1-3 in brain regions of the rat. Repeated treatment with fluoxetine, paroxetine or desipramine increased VGLUT1 mRNA abundance in frontal, orbital, cingulate and parietal cortices, and regions of the hippocampus. Immunoautoradiography analysis showed that repeated antidepressant drug treatment increased VGLUT1 protein expression. Repeated electroconvulsive shock (ECS) also increased VGLUT1 mRNA abundance in regions of the cortex and hippocampus compared to

sham controls. The antidepressant drugs and ECS did not alter VGLUT1 mRNA abundance after acute administration, and no change was detected after repeated treatment with the antipsychotic agents, haloperidol and chlorpromazine. In contrast to VGLUT1, the different antidepressant treatments did not commonly increase the expression of VGLUT2 or VGLUT3 mRNA. These data suggest that a course of antidepressant drug or ECS treatment increases expression of VGLUT1, a key gene involved in the regulation of glutamate secretion.

PMID: 15992385 [PubMed - indexed for MEDLINE]

6: J Neurosci. 2005 Jun 29;25(26):6221-34. Related Articles, Links
Presynaptic regulation of quantal size by the vesicular glutamate transporter VGLUT1.

Wilson NR, Kang J, Hueske EV, Leung T, Varoqui H, Murnick JG, Erickson JD, Liu G.

Department of Brain and Cognitive Sciences, Picower Center for Learning and Memory, and The Institute of Physical and Chemical Research (RIKEN), Massachusetts Institute of Technology, Cambridge, Massachusetts 02139-4307, USA.

A fundamental question in synaptic physiology is whether the unitary strength of a synapse can be regulated by presynaptic characteristics and, if so, what those characteristics might be. Here, we characterize a newly proposed mechanism for altering the strength of glutamatergic synapses based on the recently identified vesicular glutamate transporter VGLUT1. We provide direct evidence that filling in isolated synaptic vesicles is subject to a dynamic equilibrium that is determined by both the concentration of available glutamate and the number of vesicular transporters participating in loading. We observe that changing the number of vesicular transporters expressed at hippocampal excitatory synapses results in enhanced evoked and miniature responses and verify biophysically that these changes correspond to an increase in the amount of glutamate released per vesicle into the synaptic cleft. In addition, we find that this modulation of synaptic strength by vesicular transporter expression is endogenously regulated, both across development to coincide with a maturational increase in vesicle cycling and quantal amplitude and by excitatory and inhibitory receptor activation in mature neurons to provide an activity-dependent scaling of quantal size via a presynaptic mechanism. Together, these findings underscore that vesicular transporter expression is used endogenously to directly regulate the extent of glutamate release, providing a concise presynaptic mechanism for controlling the quantal efficacy of excitatory transmission during synaptic refinement and plasticity.

PMID: 15987952 [PubMed - in process]

7: J Neurosci Res. 2005 Aug 15;81(4):506-21. Related Articles, Links
Frequent coexpression of the vesicular glutamate transporter 1 and 2 genes, as well as coexpression with genes for choline acetyltransferase or glutamic acid decarboxylase in neurons of rat brain.

Danik M, Cassoly E, Manseau F, Sotty F, Mouginot D, Williams S.

Douglas Hospital Research Centre, McGill University, Montreal, Quebec,

Canada.

It is widely believed that expression of the vesicular glutamate transporter genes VGLUT1 and VGLUT2 is restricted to glutamatergic neurons and that the two transporters segregate in different sets of neurons. Using single-cell multiplex RT-PCR (sc-RT-mPCR), we show that VGLUT1 and VGLUT2 mRNAs were coexpressed in most of the sampled neurons from the rat hippocampus, cortex, and cerebellum at postnatal Day (P)14 but not P60. In accordance, changes in VGLUT1 and VGLUT2 mRNA concentrations were found to occur in these and other brain areas between P14 and P60, as revealed by semiquantitative RT-PCR and quantitated by ribonuclease protection assay. VGLUT1 and -2 coexpression in the hippocampal formation is supported further by in situ hybridization data showing that virtually all cells in the CA1-CA3 pyramidal and granule cell layers were highly positive for both transcripts until P14. It was revealed using sc-RT-mPCR that transcripts for VGLUT1 and VGLUT2 were also present in neurons of the cerebellum, striatum, and septum that expressed markers for gamma-aminobutyric acid (GABA)ergic or cholinergic phenotypes, as well as in hippocampal cells containing transcripts for the glial fibrillary acidic protein. Our study suggests that VGLUT1 and VGLUT2 proteins may often transport glutamate into vesicles within the same neuron, especially during early postnatal development, and that they are expressed widely in presumed glutamatergic, GABAergic, and cholinergic neurons, as well as in astrocytes. Furthermore, our study shows that such coexpressing neurons remain in the adult brain and identifies several areas that contain them in both young and adult rats. 2005 Wiley-Liss, Inc.

PMID: 15983996 [PubMed - in process]

8: Biol Pharm Bull. 2005 Jun;28(6):990-3. Related Articles, Links
Release of endogenous glutamate by AMPA receptors expressed in cultured rat costal chondrocytes.

Wang L, Hinoi E, Takemori A, Yoneda Y.

Laboratory of Molecular Pharmacology, Division of Pharmaceutical Sciences, Kanazawa University Graduate School of Natural Science and Technology, Ishikawa, Japan.

We have previously demonstrated the release of endogenous glutamate by activation of DL-alpha-amino-3-hydroxy-5-methylisoxasole-4-propionate (AMPA) receptors expressed by bone, while there is no information available on the possible functional expression of glutamatergic signaling molecules in cartilage to date. In rat costal chondrocytes cultured for 4 to 28 d, expression of mRNA was seen for several chondral marker genes including sox9, runt-related gene 2/core binding factor alpha-1 (Runx-2/Cbfa-1), type II collagen and aggrecan, but not for the adipocyte marker gene peroxisome proliferator-activated receptor gamma (PPARgamma). Expression of mRNA was drastically increased for Runx-2/Cbfa-1 during culturing from 7 to 14 d with a gradual increase thereafter up to 28 d, while a transient increase was seen in mRNA expression for both type-II collagen and sox-9 on 14 d and for aggrecan on 7 d respectively, in chondrocytes cultured for a period up to 28 d. Irrespective of the culture period up to 21 d, marked expression was seen by cultured chondrocytes with mRNA for GluR3 subunit of AMPA receptors, in addition to vesicular glutamate transporter-1 (VGLUT1) required for the condensation and subsequent exocytotic release of glutamate in the glutamatergic neurotransmission in the brain. Cultured rat costal

chondrocytes underwent spontaneous release of endogenous glutamate, while an inhibitor of AMPA receptor desensitization significantly prolonged the duration of endogenous glutamate release stimulated by AMPA. These results suggest that endogenous glutamate could be released from intracellular vesicular constituents associated with VGLUT1 through activation of AMPA receptors expressed by cultured rat costal chondrocytes.

PMID: 15930732 [PubMed - in process]